American Journal of Medical Genetics 68:231–235 (1997)

Hypertelorism and Hypospadias Associated With a De Novo Apparently Balanced Translocation Between 8q22.3-23 and 20p13

A. Tar,[1] A. Ion,[2] J. Sólyom,[3] B. Györvári,[3] C. Stephenson,[4] S. Barbaux,[5] M. Nunes,[5] M. Fellous,[5] and K. McElreavey[5*]

[1]*Buda Children's Hospital, Budapest, Hungary*
[2]*Laboratoire de Cytogénétique Constitutionelle, Hôpital St. Vincent de Paul, Paris, France*
[3]*Second Department of Pediatrics, Semmelweis University, Budapest, Hungary*
[4]*Oncogenetics, Phoenix, Arizona*
[5]*Immunogénétique Humaine, Institut Pasteur, Paris Cedex, France*

A de novo apparently balanced translocation involving chromosomes 8 and 20 was found in a 14-year-old boy with minor anomalies, mild skeletal abnormalities and ambiguous external genitalia including perineoscrotal hypospadias, rudimentary fused labioscrotal folds, bilateral cryptorchidism, and small penis. The karyotype was 46,XY, t(8;20)(q22.3-23;p13). No signs of other conditions known to be associated with structural anomalies of either chromosome 8 or 20 were present and incomplete masculinisation of the external genitalia appears to be the main component of the phenotype. Clinical and biological studies showed apparently normal testicular function in utero and after birth. Examinations excluded 5α-reductase deficiency or a block in any enzymatic steps of testosterone, glucocorticoid and mineralocorticoid biosynthesis. Coding sequences of the sex-determining gene (SRY) and androgen receptor gene (AR) were found to be identical to those of a normal male excluding their role in the cause of the present condition. Since several other reports describe the association of hypospadias and hypertelorism with deletions or translocations involving 8q, we suggest that a locus necessary for male sex differentiation is located at distal 8q. Am. J. Med. Genet. 68:231–235, 1997 © 1997 Wiley-Liss, Inc.

KEY WORDS: hypospadias; hypertelorism; chromosome 8; sex differentiation

INTRODUCTION

In 46,XY individuals, the formation of testis is the primary and essential event in the differentiation of the male genital phenotype by the subsequent testicular production of male sex steroids and anti-Müllerian hormone. Correct testicular development is dependent on the activity of the SRY gene which is located on Yp [Sinclair et al., 1990]. Absence or mutations in this gene result in abnormal male sexual development ranging from ambiguous external genitalia to complete sex reversal [McElreavey et al., 1992 and unpublished observations]. Abnormal sexual differentiation in XY individuals may have a wide variety of other causes including androgen resistance, defects in steroid biosynthesis and may occur in association with several complex clinical conditions such as Denys-Drash syndrome, campomelic syndrome and X-linked α-thalassemia with mental retardation [ATR-X] syndrome [Pelletier et al., 1991; Foster et al., 1994; Gibbons et al., 1995]. Recently, several chromosome abnormalities have been reported to be associated with ambiguous or female genitalia in 46,XY subjects including Xp duplications and monosomy of portions of 9p and 10q [Bardoni et al., 1993; Bennett et al., 1993; Wilkie et al., 1993]. This suggests that several loci are involved in normal male sexual differentiation.

Embryological studies showed that male and female external genitalia and the urethra develop from the genital tubercle, the genital folds and the urogenital sinus. The differentiation of those primary structures into male internal and external genitalia requires the presence of testosterone and its conversion to dihydrotestosterone by 5α-reductase. The specific response to both of these hormones is mediated through the intranuclear androgen receptor, a transcriptional regulator of the steroid hormone receptor superfamily [Lubahn et al., 1988]. Errors occurring at any step of this hormonal pathway may lead to abnormalities in the development of the male genital phenotype, one of the most frequent of which is hypospadias [8 in 1,000 live births, Roberts and Lloyd, 1973]. In this disorder the position of the opening of the urethral orifice is located on the ventral side of the penis rather than at the

Contract grant sponsor: INSERM; Contract grant sponsor: AFM; Contract grant sponsor: La Recherche Medicale

*Correspondence to: Ken McElreavey, Immunogénétique Humaine, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris Cedex 15, France.

Received 11 November 1995; Accepted 2 May 1996

tip of the glans. Hypospadias has been reported to be caused by rare defects in testosterone biosynthesis [Savage, 1990] and two familial cases of hypospadias have been reported to be associated with mutations in the androgen receptor gene [Batch et al., 1993; Hiort et al., 1994]; however, in most cases the cause of the disorder is unknown. In several cases hypospadias was reported in association with other congenital abnormalities including hypertelorism [Opitz, 1969] or polysyndactyly [Naguib, 1988].

We report on a 46,XY individual with hypospadias, cryptorchidism, hypertelorism, minor anomalies and an apparently balanced translocation between chromosomes 8 and 20: 46,XY,t(8;20)(q22.3-23;p13). On the basis of this case, together with 3 other previously published reports, we suggest that a locus necessary for the development of male external genitalia is located at distal 8q.

MATERIALS AND METHODS

Cytogenetic Analysis

Cytogenetic analysis was performed on peripheral blood from the patient and his normal parents. Lymphocytes were cultured according to a standard method. Banding patterns were analysed using RHG, GTG and CTG techniques as described previously. QFQ staining was performed according to Caspersson et al. [1970].

Fluorescent in situ hybridisation (FISH) was performed using a chromosome 8 (biotin-labelled) and chromosome 20 (digoxigenin-labelled) specific library probe supplied by ONCOR (Gaithersburg, MD). The in situ hybridisation was performed on metaphases from EBV-transformed lymphoblastoid cells.

DNA Analysis

Southern blotting of genomic DNA from the patient was performed using the probe pY53.3 [which contains the testis determining gene, SRY; Sinclair et al., 1990]. In addition, coding sequences of the SRY and AR genes were screened for the presence of mutations using two polymerase chain reaction (PCR)-based methods, denaturing gradient gel electrophoresis (DGGE) and single strand conformational polymorphism (SSCP), respectively. Details of both procedures have been described elsewhere [McElreavey et al., 1992; Lobaccaro et al., 1993].

Clinical Report

The patient was born on November 10, 1981 to a 24-year-old gravida 2 woman and her phenotypically normal husband. Both parents were of Hungarian origin and no evidence of consanguinity was found in the family history. Pregnancy and delivery were uneventful. Birth weight and length were 2,000 g and 46 cm, respectively.

At birth, the child presented with ambiguous external genitalia, minor anomalies, subluxation of the index and clinodactyly of the 5th finger of the right hand. At 2 weeks he had a 2-cm-long penis with perineoscrotal hypospadias, rudimentary and partially fused labioscrotal folds, bilateral cryptorchidism, hypertelorism (inner canthal distance 26 mm, outer canthal distance 114 cm, inner canthal index 5.5), convergent strabismus and apparently low-set ears (Fig. 1). At this time his serum testosterone level was 1.82 ng/ml (normal 0.5–8 ng/ml), serum 17-OH progesterone: 1.25 ng/ml (normal 0.3–2.6 ng/ml), DHEAS: 0.15 ng/ml and serum cortisol <2.5 µg/dl.

At age 3 years the child underwent bilateral orchidopexy and repair of the hypospadias. No histological examination was performed on the gonads. Hormonal investigations were consistent with normal testicular function, since serum testosterone was 6.6 ng/ml and rose to 60.7 ng/ml after 3,000 IU hCG stimulation (within the normal range).

At 7 years, abdominal ultrasound study showed a 3×1 cm blind vaginal-like pouch connected to the proximal part of the urethra. At age 12 years, the child was short (height 135.8 cm, <2 S.D. and weight 28.9 kg, <2 S.D.), without evidence of mental retardation or other somatic anomalies. At that time, serum FSH level was 7.7 IU/l rising to 70 IU/l after LHRH stimulation, serum LH: 18,8 IU/l rising to 43 IU/l, serum testosterone was 2.32 ng/ml and serum DHT 0.8 ng/ml (ratio 2.9), indicating a normal 5α-reductase activity. After arginine-TRH stimulation, serum growth hormone levels rose from 0.56 ng/ml to 40 ng/ml (normal >10 ng/ml), serum TSH: rose from 1.75 to 13 µU/ml (serum T4 was 5.4 µg/dl) thereby excluding a dysfunction of the hypothalamic-hypophyseal axis.

RESULTS

Karyotype analysis of the subject using RHG and GTG banding showed a de novo 46,XY,t(8;20)(q22.3-23;p13) chromosome complement (Fig. 2). Father and mother had normal chromosomes. Twenty QFQ-banded metaphases were counted for the patient and showed a normally fluorescent Y chromosome. The (8;20) de novo balanced translocation was confirmed using the fluorescence in situ hybridisation technique (data not shown). The position of the breakpoints is indicated in the schematic representation (Fig. 3).

To exclude a role for the testis determining gene SRY and the androgen receptor (AR) gene in the cause of the abnormal phenotype, DGGE and SSCP analysis was

Fig. 1. The face of the patient at 11 years showing hypertelorism.

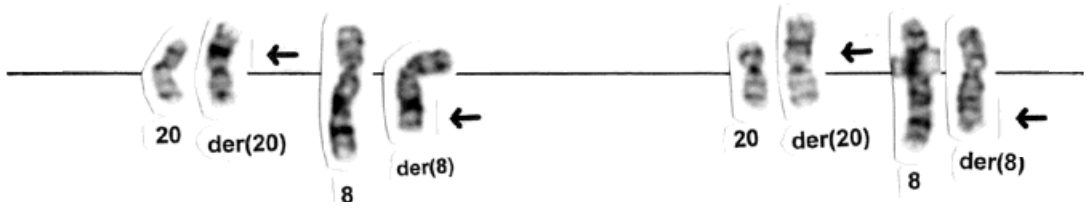

Fig. 2. Karyotype of the patient using high resolution banding. The rearranged chromosomes 8 and 20 are indicated by arrows.

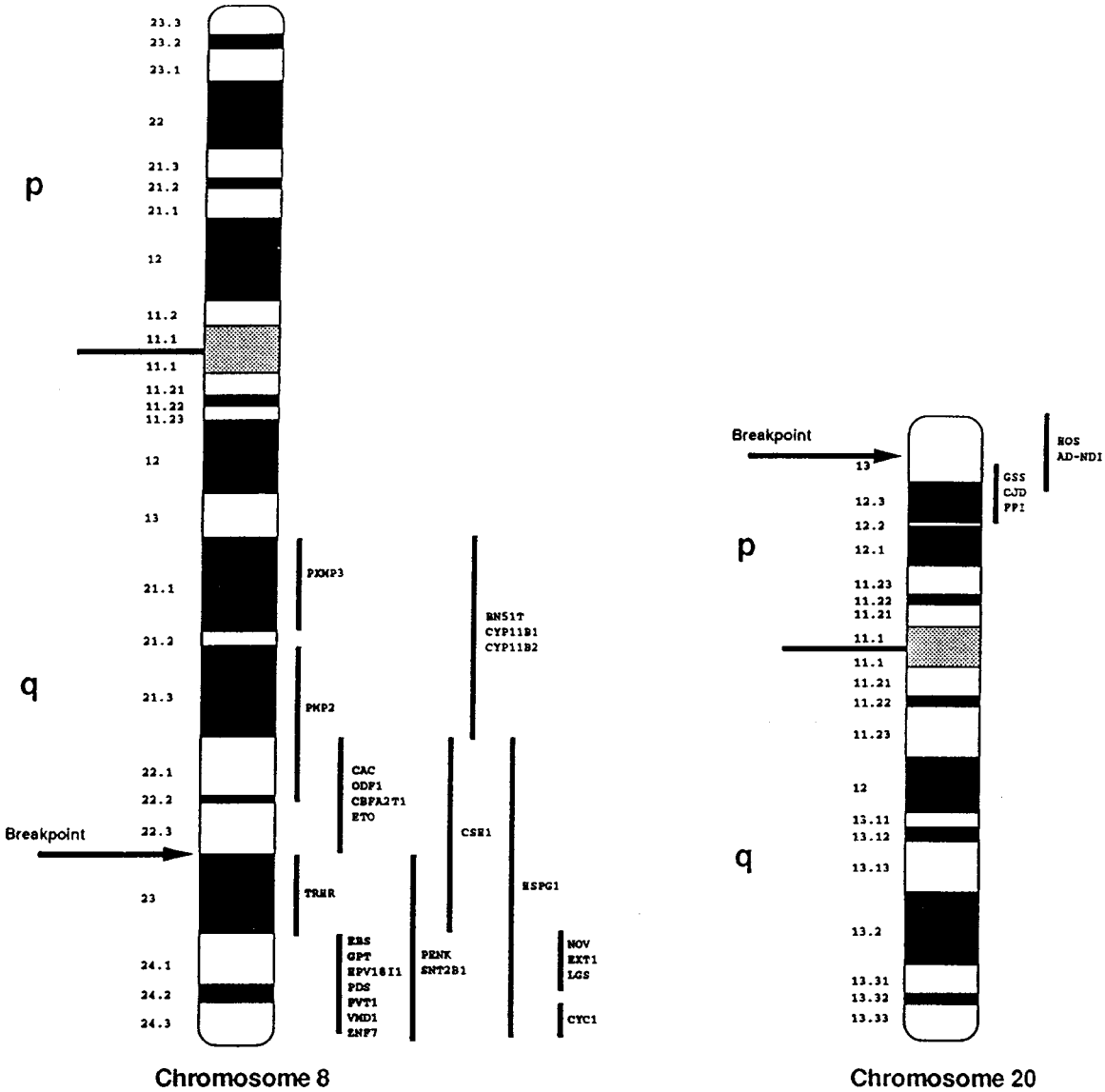

Fig. 3. Schematic representation of chromosomes 8 and 20 indicating the position of the breakpoints in the patient. The order and cytogenetic positions of loci and genes in the immediate vicinity are indicated. Details of the genes and loci are available from the Genome Data Base.

performed. Coding sequences of both genes were found to be identical to that of a normal control male (data not shown). Southern blotting of genomic DNA using the probe pY53.3 showed a hybridisation pattern identical to that of a normal male (data not shown).

DISCUSSION

The boy described here had normal male internal genitalia and lacked Müllerian structures which indicates that Sertoli and Leydig cell function was normal in utero. Serum testosterone and gonadotropin levels were normal at birth and during infancy; hence, testicular function appeared to be normal. These data, together with a wild-type SRY gene, suggest that both gonad determination and differentiation were appropriate. An abnormality of androgen production or function in the case described in this paper can be excluded since coding sequences of the AR gene were found to be identical to a normal male and serum androgen levels were not elevated.

Although the patient described here had a vaginal pouch-like structure, an abnormality of dihydrotestosterone seems unlikely since the testosterone : dihydrotestosterone ratio was not elevated.

Hypertelorism and hypospadias is found in several syndromes, including the G or BBB syndrome (now termed the Opitz GBBB syndrome) [Opitz, 1969] and acrofrontofacionasal (AFFN) syndrome [Naguib, 1988]. The clinical expression of Opitz GBBB syndrome is variable even within a family; however, a constellation of midline defects, including hypertelorism, esophageal dysmotility, cleft palate and, in males, hypospadias, are common manifestations. Hypertelorism, hypospadias and polysyndactyly are the clinical findings of the rarer AFFN syndrome. Pedigree analysis indicates that in many cases the transmission of Opitz GBBB syndrome is consistent with an autosomal dominant mode of inheritance, whereas the parental consanguinity noted in familial cases of the AFFN syndrome suggests autosomal recessive inheritance [Naguib, 1988]. Recently, the Opitz GBBB syndrome was mapped to Xp22 and 22q11.2, showing that the syndrome is genetically heterogeneous [Robin et al., 1995]. Hypospadias and hypertelorism are the only midline defects characteristic of the Opitz GBBB syndrome present in the patient described in this paper; however, formally it cannot be excluded that he has a mild form of Opitz GBBB syndrome.

The possibility that his minor anomalies were a consequence of the de novo translocation between chromosomes 8 and 20 seems probable. Rearrangements of chromosome 20 have not been described in cases with hypertelorism-hypospadias, although some individuals with a ring 20 chromosome and cryptorchidism have been reported [Porfirio et al., 1987 and references therein]. Abnormal development of external genitalia in 46,XY individuals with hypertelorism was reported in several males who had a partial monosomy of distal 8q. Taysi et al. [1979] described a male infant who presented with psychomotor retardation and multiple congenital abnormalities including small phallus with mild hypospadias, undescended testes and marked hypertelorism. An interstitial deletion of 8q13-q22 was described. In 1983 Rivera et al. reported on a 46,XY male with a de novo deletion of 8q21.2-q22 and phenotype similar to that described by Taysi et al. [1979]. The genitalia were small and the patient had cryptorchidism, hypertelorism and clinodactyly of the fifth fingers. Say and Carpenter [1987] also described a male infant with multiple congenital abnormalities, first-degree hypospadias, hypertelorism, cleft lip and palate, imperforate anus and mental retardation, leading the authors to suggest that he may have had the BBB syndrome. Cytogenetic investigations demonstrated a complex balanced translocation with a breakpoint at 8q22. The complete karyotype was 46,XY,t(5;8;10)(5pter-5q13::8q22-8qter;8pter-8q22::10q11-10qter::10pter-10q11::5q13-5qter). Chromosomal monosomy either immediately proximal or distal to this region is associated with two clinical syndromes: branchio-oto-renal syndrome [BOR; 8q12-q21.2; Vincent et al., 1994] and Langer-Giedion syndrome [LGS; 8q24.1; Bowen et al., 1985]. Neither hypospadias nor hypertelorism occurs in either of these 2 syndromes. The absence of manifestations typical of BOR or LGS in our subject and the 3 cases described above, together with the remarkable phenotypic similarity between these 4 cases, leads us to speculate that there is a locus at 8q22.3-23 which when disrupted causes hypertelorism and hypospadias.

ACKNOWLEDGMENTS

The authors thank Nicole Souleyreau for excellent technical assistance. This work was supported by INSERM, AFM and Foundation pour La Recherche Medicale.

REFERENCES

Bardoni B, Florida G, Guioli S, Peverali G, Anichini C, Cisterino M, Casalone R, Danesio C, Fracarro M, Zuffardi O, Camerino G (1993): Functional disomy of Xp22-pter in 3 males carrying a portion of Xp translocated to Yq. Hum Genet 91: 333–338.

Batch JA, Evans BAJ, Hughes IA, Patterson MN (1993): Mutation of the androgen receptor gene in perineal hypospadias. J Med Genet 30: 198–201.

Bennett CP, Docherty Z, Robb SA, Ramani P, Hawkins JR, Grant D (1993): Deletion 9p and sex reversal. J Med Genet 30: 518–520.

Bowen P, Biederman B, Hoo JJ (1985): The critical segment for the Langer-Giedion syndrome: 8q24.11-q24.12 Ann Génét 28: 224–227.

Caspersson T, Zech L. Johansson C (1970): Differential binding of alkylating fluorochromes in human chromosomes. Exp Cell Res 60: 315–319.

Foster JW, Dominquez-Steglich MA, Guioli S, Kwok C, Weller PA, Stevanic M, Weissenbach J, Mansour S, Young ID, Goodfellow PN, Brook JD, Schafer A (1994): Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. Nature 372: 525–530.

Gibbons RJ, Picketts DJ, Villard L, Higgs DR (1995): Mutations in a putative global transcriptional regulator cause X-linked mental retardation with a-thalassemia (ATR-X syndrome). Cell 80: 837–845.

Hiort O, Klauber G, Cendron M, Sinnecker GHG, Keim L, Schwinger E, Wolfe HJ, Yandell DW (1994): Molecular characterisation of the androgen receptor gene in boys with hypospadias. Eur J Ped 153: 317–321.

Lobaccaro JM, Lumbroso S, Berta P, Chaussain JL, Sultan C (1993): Complete androgen insensitivity syndrome associated with a *de novo* mutation of the androgen receptor gene detected by single

strand conformation polymorphism. J Steroid Biochem Mol Biol 44: 211–216.

Lubahn D, Joseph DR, Sullivan PM, Williard HF, French FS, Wilson EM (1988): Cloning of the human androgen receptor complementary DNA and localisation to the X chromosome. Science 240: 327–330.

McElreavey K, Vilain E, Nacer A, Costa J-M, Souleyreau N, Kucheria K, Boucekkine C, Thibaud E, Brauner R, Flamant F, Fellous M (1992): XY sex reversal associated with a deletion 5′ to the SRY "HMG box" in the testis-determining region. Proc Natl Acad Sci (USA) 89: 11016–11020.

Naguib KK (1988): Hypertelorism, proptosis, ptosis, hypospadias and normal height in 3 sibs: A new syndrome? Am J Med Genet 29: 35–41.

Opitz, JM (1969): The G syndrome of multiple congenital abnormalities. Birth Defects Orig Art Ser V (2): 95–101.

Pelletier J, Bruening W, Kashtan CE, Mauer SM, Manivel JC, Striegel JE, Houghton DC, Junien C, Habib R, Fouser L, Fine RN, Silverman BL, Haber DA, Housman D (1991): Germline mutations in the Wilms' tumor suppressor gene are associated with abnormal urogenital development in Denys-Drash syndrome. Cell 67:437–447.

Porfirio B, Valorani M, Giannotti A, Sabetta G, Dallapiccola B (1987): Ring 20 chromosome phenotype. J Med Genet 24: 375–377.

Riviera H, Rodriguez RM, Plascencia ML, Martinez y Martinez R, Nazara, Z, Cantú JM (1983): Del(8)(q212q2200) *de novo* in a boy without Langer-Giedion syndrome. J Génét Hum 31: 413–418.

Roberts C, Lloyd S (1973): Observation on the epidemiology of simple hypospadias. Br Med J 1:768–770.

Robin NH, Feldman GJ, Aronson AL, Mitchell HF, Weksberg R, Leonard CO, Burton BK, Josephson KD, Laxova R, Aleck KA, Allanson JE, Guion-Almeida, Martin RA, Leichtman LG, Price RA, Opitz JM, Muenke M (1995): Opitz syndrome is genetically heterogeneous, with one locus on Xp22, and a second locus at 22q11.2. Nat Genet 11:459–461.

Savage M (1989): Clinical aspects of intersex. In. Book C (ed): "Clinical Paedriatic Endocrinology." 2nd ed. Oxford: Blackwell Scientific Publications, pp 38–54.

Say B, Carpenter NJ (1987): Skeletal dysplasia in an infant with hypertelorism, hypospadias, developmental delay, and a complex chromosomal translocation. South Med J 80: 1190–1192.

Sinclair AH, Berta P, Palmer M, Hawkins JR, Griffiths BL, Smith MJ, Foster JM, Frischauf AM, Lovell-Badge R, Goodfellow PN (1990): A gene from the human sex determining region encodes a protein with homology to a conserved DNA binding motif. Nature 346: 240–244.

Taysi K, Noetzel MJ, Strauss AW (1979): Presumptive long arm deletion of chromosome 8: A new syndrome? Hum Genet 51: 49–53.

Vincent C, Kalatzis V, Compain S, Levilliers J, Slim R, Graia F, Pereira ML, Nivelon A, Croquette MF Lacombe D, Vigeron J, Helias J, Broyer M, Callen DF, Haan EA, Weissenbach J, Lacroix B, Bellane-Chantelot C, Le Paslier D, Cohen D, Petit C (1994): A proposed new contiguous gene syndrome on 8q consists of branchio-oto-renal (BOR) syndrome, Duane syndrome, a dominant form of hydrocephalus and trapezeius? aplasia; implications for the mapping of the BOR gene. Hum Mol Genet 3: 1859–1866.

Wilkie AO, Campbell FM, Daubeney P, Grant DB, Daniels RJ, Mullarkey M, Affara NA, Fitchett M, Huson SM (1993): Complete and partial XY sex reversal associated with terminal deletion of 10q: Report of two cases and literature review. Am J Med Genet 46: 597–600.